Morphogenesis of trichomes of *Pelargonium scabrum*

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The indumentum of the lamina of *Pelargonium scabrum* (L.) L'Hérit. consists of only two types of trichomes, i.e. five-celled glandular hairs which produce essential oils and unicellular spiny hairs which are basally surrounded by enlarged epidermal cells. Every glandular hair consists of a globular head, three stalk cells and a characteristically elongated basal cell. Both glandular and spiny hairs develop by enlargement and/or division of a single epidermal cell. Ontogenetio studies have indicated that the different morphological types of glandular hairs in *P. scabrum* represent different developmental stages of a single glandular hair type. Secretion of essential oils apparently occurs repeatedly from the young five-celled stage, as a new cuticle develops time and again beneath the ruptured one.
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Die indumentum van die lamina van *Pelargonium scabrum* (L.) L'Hérit. bestaan uit slegs twee tipes trigome, nl. vyfsellige klierhare wat essensiële olies produseer en eensellige stekelhare wat basaal deur vergrote epidermisselle omring word. Die klierhare bestaan elk uit 'n bolvormige kop, driesellige steel en kenmerkende verlengde basale sel. Belde die klierhare en stekelhare ontwikkel deur vergroting en/of verdeling van 'n enkele epidermissel. Daar is ontogeneties vasgestel dat die verskillende morfologiese klierhaartipes by *P. scabrum* bloot verskillende ontwikkelingstadiums van 'n enkele klierhaartipe verteenwoordig. Sekresie van essensiële olies vind skynbaar vanaf die jong vyfsellige stadium plaas, deurdat 'n nuwe kutikula telkens onder die oopgebarste een gevorm word.
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Introduction

Trichomes are epidermal appendages of diverse form, structure and function (Uphof 1962). Despite the variety of systems that exist for the classification of trichome types, they are ultimately classified as being either glandular with a secretory function, or covering hairs (non-glandular) without a secretory function (Cutter 1978).

The development of trichomes from the epidermis results from differential enlargement and subsequent divisions of epidermal cells and their derivatives (Carlquist 1958). In his classification of different trichome types, Uphof (1962) used the plane of division of the initial epidermal cell as a distinctive characteristic. Where more than one trichome type occurs in a single species, each apparently has a special developmental pathway, as the different structural forms are not one type which is arrested at different stages in a common pathway (Hammond & Mahlberg 1973).

Pelargonium L'Hérit species are often conspicuously hairy and aromatic (van der Walt 1977). The trichome types occurring in the various species of *Pelargonium* have already proved to be of taxonomic value (Oosthuizen 1983). The essential oils produced by the glandular hairs, furthermore, are of economic importance in the perfume industry (Schery 1972). Despite the significance of the trichomes of *Pelargonium* little is known about their initiation and development. It is also possible that in the classification of trichome types in *Pelargonium* (Oosthuizen 1983), different developmental stages of a trichome have been classified as different trichome types.

The aim of this paper is to describe the morphogenesis of the trichomes of *Pelargonium scabrum* (L.) L'Hérit., with special emphasis on the structure of the glandular hairs in their different developmental stages. The paper represents the initial investigation of the developmental and functional relationships between glandular hairs and the biogenesis of essential oils in *Pelargonium*.

Material and Methods

Specimens were prepared from cuttings of a single individual of *P. scabrum* to ensure the use of genetically identical material throughout the study. The mother plant was collected in the veld near Stellenbosch and grown in the Botanical Gardens of the University of Stellenbosch.

Because the young leaves have a denser indumentum than the older ones, only the uppermost leaves were prepared for microscopic investigation. This ensures that abundant trichomes will be present in the small pieces of plant material prepared for electron microscopy.

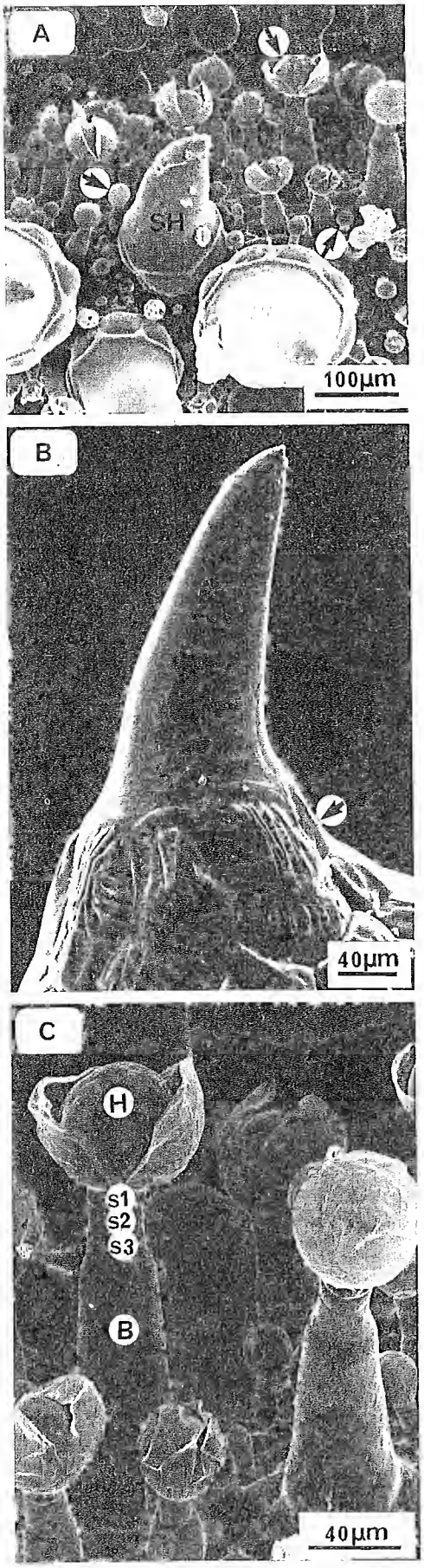

Figure 1 Scanning electron micrographs of trichomes of the indumentum of the lamina of *P. scabrum*. A. Indumentum, consisting of spiny hairs (SH) and glandular hairs of various morphological forms (arrows); B. Spiny hair with swollen base surrounded by podium cells (arrow); C. Mature glandular hair, consisting of a unicellular head (H), three stalk cells (S1, S2 & S3) and an elongated basal cell (B).

Light and transmission electron microscopy

Material was fixed at room temperature for 3 h in 3% glutaraldehyde (Merck) in 0,05 mol dm^{-3} cacodylate buffer (pH 7,2), washed in a solution of 0,05 mol dm^{-3} cacodylate buffer (pH 7,2) in 0,34 mol dm^{-3} sucrose, post-fixed for 1 h in 1% OsO_4 in 0,05 mol dm^{-3} cacodylate buffer (pH 7,2) and 0,28 mol dm^{-3} sucrose, and washed again as before. Owing to the sucrose added during fixation the solutions all had an osmolality of 350 to 400 milliosmols. The fixed specimens were then dehydrated in a graded acetone series. To promote infiltration with Spurr's low viscosity epoxy resin (Spurr 1969), the material was put into propylene oxide for 30 min, followed by an hour in 50% resin in propylene oxide, and finally overnight in Spurr's resin. All the above-mentioned procedures were carried out at room temperature and in all steps after fixation the 8 cm^3 Polytops with fixed specimens were rotated at 1 r.p.m. to enhance infiltration. Polymerization of the specimens embedded in Spurr's resin was carried out for 18 h at 70 °C.

Semithin (0,5 µm) and ultrathin (silver to gold) sections were cut with Reichert-Jung OM U3 and OM U4 ultramicrotomes using diamond knives. The semithin sections were mounted on slides treated with a chrome alum-gelatin solution (Pappas 1971; Warmke & Lee 1976), dried at 45 °C and stained with Azure II/Methylene Blue (Richardson *et al.* 1960). Specimens were viewed with a Leitz photo-microscope and photographed with Panatomic X-film. The ultrathin sections were stretched with chloroform vapour, mounted on 200 or 300 mesh copper grids, stained for 10 min with 5% uranyl acetate and then for 10 min with lead citrate (Reynolds 1963). Sections were viewed with Siemens Elmiskop 101 and Philips EM 301 transmission electron microscopes operated at 60 kV.

Scanning electron microscopy

Specimens were collected, fixed and dehydrated as described above with the exception that only glutaraldehyde was used as fixative and that fixation was carried out for 24 h owing to the larger specimens being prepared for SEM. After dehydration specimens were critical point dried with liquid CO_2, gold coated, and viewed with a Jeol JSM-35 scanning electron microscope operated at 12 kV.

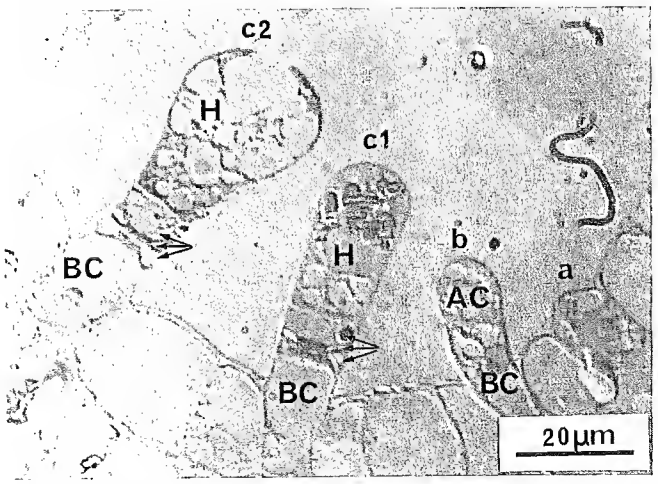

Figure 2 Light micrograph showing four stages in the development of the glandular hair of *P. scabrum*. (a. Papillate outgrowth of epidermal cell representing glandular hair initial; b. Two-celled glandular hair, consisting of an apical cell (AC) and a basal cell (BC); c1 & c2. Five-celled glandular hair, consisting of a head (H), three stalk cells (arrows) and a basal cell (BC). Initially the head is narrow and oval-shaped (c1), later enlarging to a globular shape (c2).)

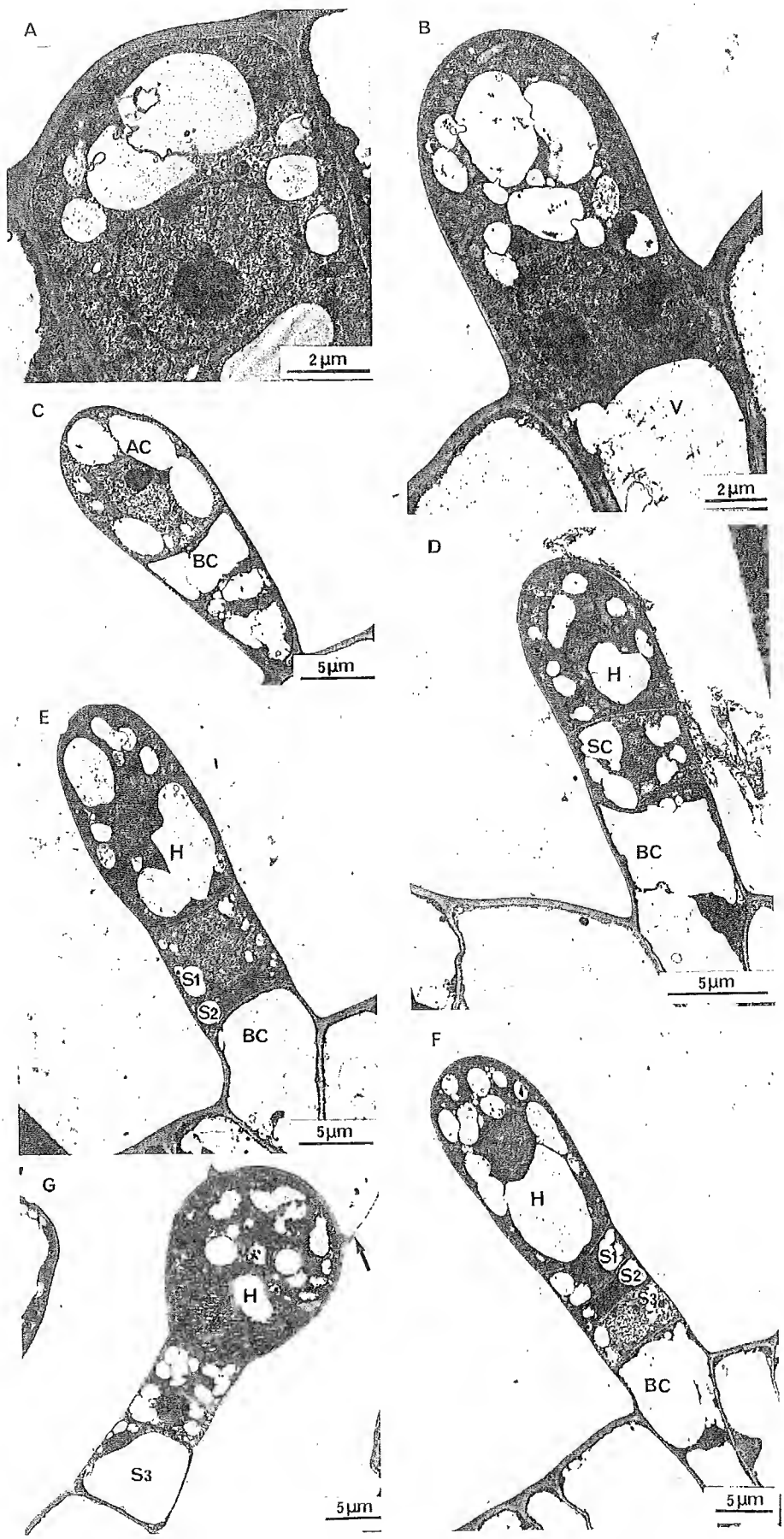

Figure 3 Transmission electron micrographs of various stages in the development of the glandular hairs of *P. scabrum*. A. Unicellular stage: papillate outgrowth of epidermal cell representing glandular hair initial. Note that the cytoplasm is dense with an apparent lack of chloroplasts; B. Elongated glandular hair initial with vacuolated basal region (V); C. Two-celled glandular hair stage, consisting of an apical cell (AC) and a basal cell (BC); D. Three-celled glandular hair stage, consisting of a head (H), stalk cell (SC) and a vacuolated, elongated basal cell (BC); E. Four-celled glandular hair stage, consisting of a head (H), two stalk cells (S1 & S2) and a vacuolated, elongated basal cell (BC); F. Five-celled glandular hair consisting of an oval-shaped head (H), three stalk cells (S1, S2 & S3) and a vacuolated, elongated basal cell (BC); G. Five-celled glandular hair (later stage than F): note that the third stalk cell (S3) is vacuolated, the head (H) has a globular shape and the cuticle has ruptured to release a secretory product (arrow).

Results

Indumentum

The indumentum of the lamina of *P. scabrum* consists of unicellular spiny hairs and glandular hairs displaying various morphological forms (Figure 1A). The spiny hairs have a swollen base surrounded by enlarged epidermal cells that constitute the characteristic podium (Figure 1B). The glandular hairs produce essential oils and in the mature state they consist of five cells, i.e. a unicellular head, a three-celled stalk and a characteristically elongated basal cell (Figure 1C).

Glandular hairs

All glandular hairs originate from papillate outgrowths of a single epidermal cell (Figures 2 & 3A). This initial is delimited from the other epidermal cells by its dense cytoplasm, and from the stoma guard cells, which also possess a dense cytoplasm, by the apparent absence of chloroplasts. The glandular hair initial elongates markedly and polarization into apical and basal parts occurs by vacuolization of the basal part of the cell (Figure 3B).

The first cell division is transverse and gives rise to a vacuolated basal cell and an apical cell with dense cytoplasm (Figures 2 & 3C). The basal cell elongates and does not take part in any further cell divisions so that the head and three stalk cells develop from the apical cell.

A transverse division of the apical cell gives rise to a three-celled glandular hair consisting of a head and stalk cell with dense cytoplasm, as well as a basal cell (Figure 3D). By a transverse division of the stalk cell a four-celled glandular hair is formed, the two middle cells being the stalk (Figure 3E). Finally the uppermost stalk cell divides transversally to give rise to a five-celled glandular hair consisting of a unicellular head and three stalk cells with dense cytoplasm, as well as a vacuolated basal cell with only a peripheral layer of cytoplasm (Figure 3F). The oval head enlarges to a globular shape while the basal cell elongates and broadens to its characteristic shape (Figures 2 & 3G). At a later stage the third stalk cell is also vacuolated (Figure 3G).

Secretion of essential oils occurs from the young five-celled stage. The young glandular head has a smooth surface but with the accumulation of oils in the subcuticular space between the cell wall and cuticle a protrusion is formed on top of the head. No pores occur in the cuticle, therefore it ruptures to release the secretory product (Figures 3G & 4A). The accumulation process is then repeated since a new cuticle is apparently formed under the ruptured one (Figures 4B & 4C). Secretion thus occurs repeatedly in young and old five-celled glandular hairs.

Observations of the repeated secretion of essential oils, despite the absence of pores in the cuticle and the rupture of the cuticle with secretion, led to the conclusion that a new cuticle must be formed repeatedly during secretion. In *Inula viscosa*, where lipids are produced continuously throughout the life of the hair, materials are secreted directly through the cell wall, without the obstacle of the cuticle, once the cuticle has ruptured (Werker & Fahn 1981). In *P. scabrum* the situation is quite different since a second protrusion of the subcuticular space with accumulated essential oils was observed with the ruptured cuticle still attached to the head (Figure 4B). Evidence of the formation of a second cuticle is also seen in Figure 4C. In the literature no evidence of similar observations could be found and this phenomenon may have implications in the initiation of the cell wall and cuticle.

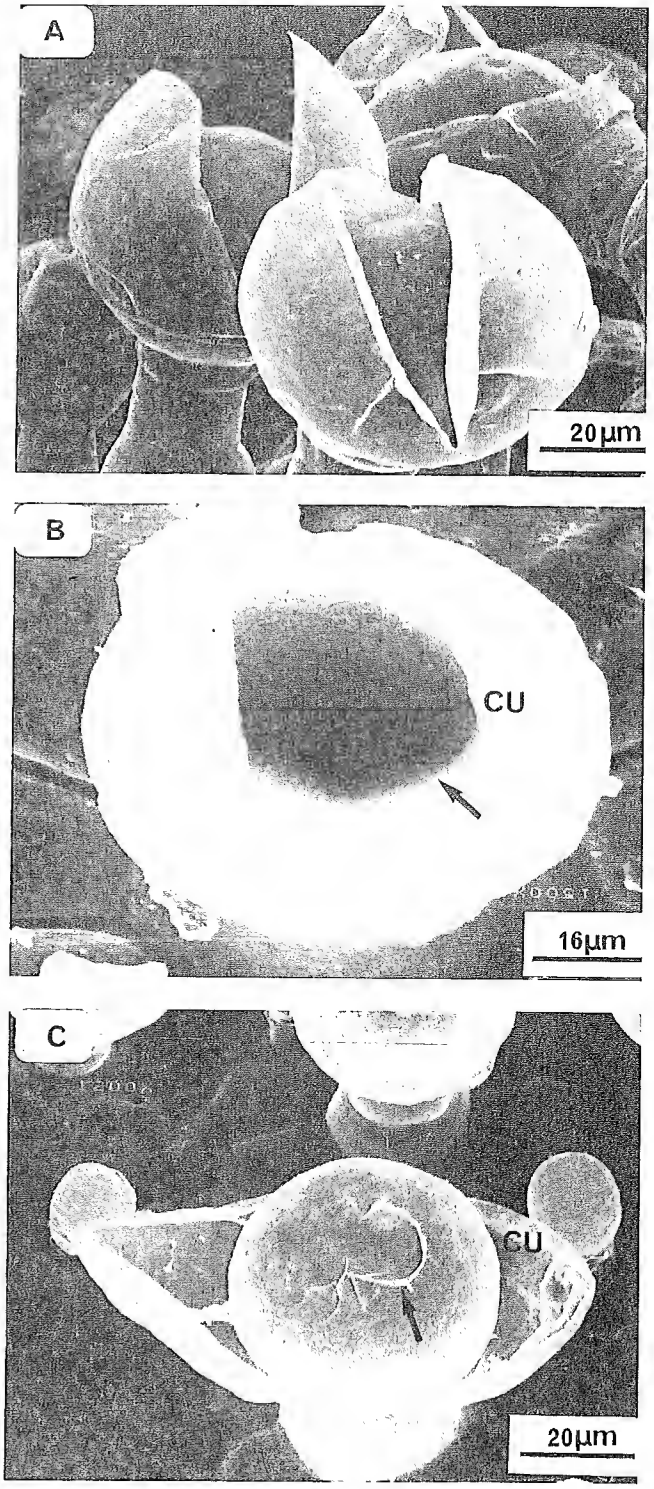

Figure 4 Scanning electron micrographs showing stages in the secretory process of glandular hairs of *P. scabrum*. A. Glandular head showing ruptured cuticle; B. Glandular head with secretory product accumulated under new cuticle (arrow). Previously ruptured cuticle (CU) still present; C. Glandular head with evidence of formation of second cuticle (arrow) under ruptured cuticle (CU).

Spiny hairs

Spiny hairs are also initiated as a protrusion of epidermal cells, although the size of these protrusions exceed that of the glandular hairs considerably. The epidermal cells that will give rise to the podium can be distinguished from an early stage as enlarged cells surrounding the spiny hair initial. These initials enlarge rapidly to give rise to swollen, oval structures. The above-mentioned stages of development are all illustrated in Figure 5A.

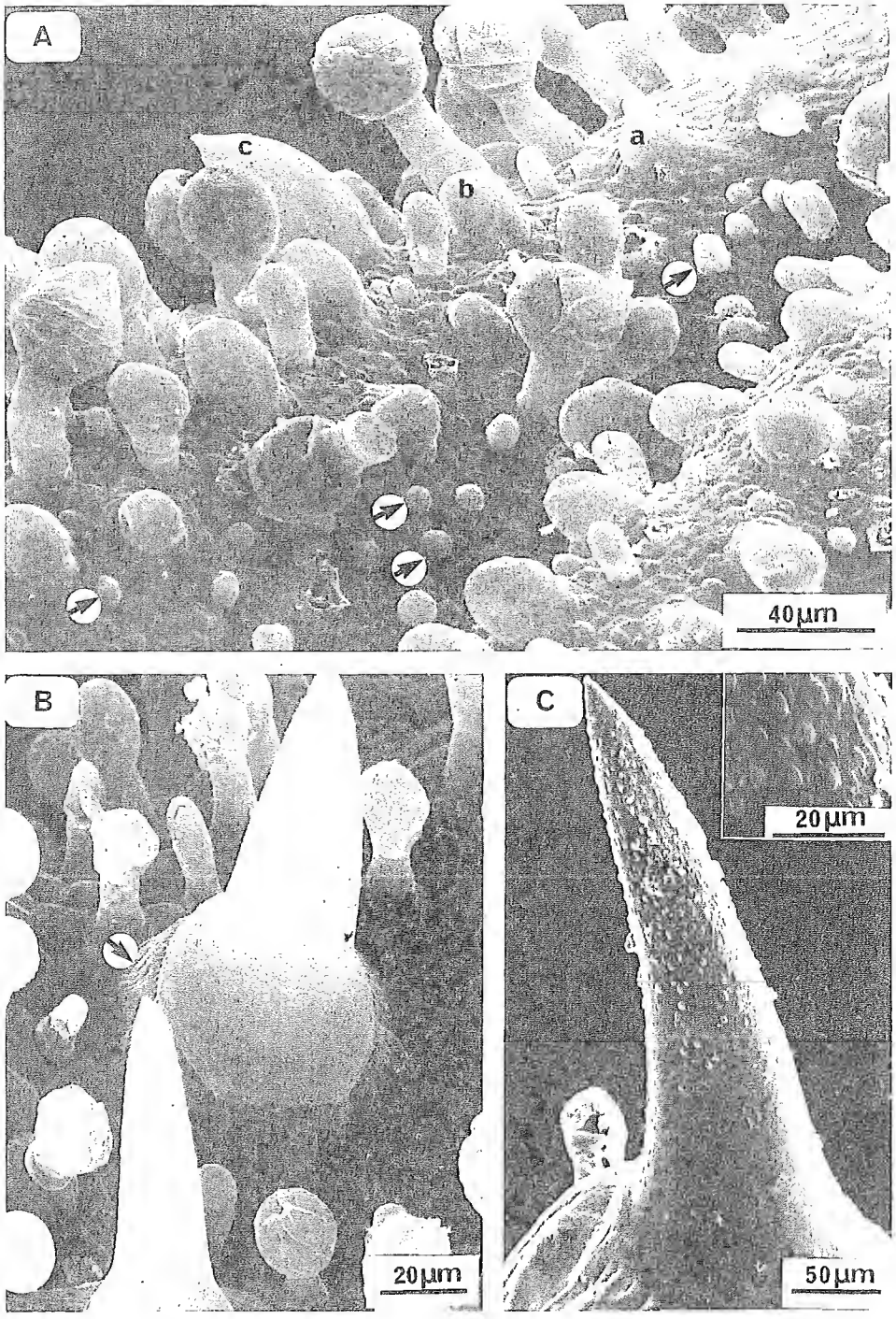

Figure 5 Scanning electron micrographs of stages in the development of the spiny hair of *P. scabrum*. A. Initials of spiny hairs (a, b & c) and glandular hairs (arrows). (a. Epidermal outgrowth representing spiny hair initial; b. Spiny hair initial surrounded by enlarged epidermal cells forming the podium; c. Swollen, oval structure of young spiny hair); B. A developing spiny hair raised above the leaf surface with the base surrounded by enlarged podium cells (arrow); C. A mature spiny hair with warty surface sculpture. Inset is a micrograph of warts at higher magnification.

Subsequently the spiny hair narrows distally to a sharp point, the hair base and podium cells enlarge (Figure 5B) and the spiny hair is lifted above the leaf surface with the base sunk into the podium (Figure 1B). The mature spiny hair sometimes develops a warty surface sculpture that extends from the top to the base of the hair (Figure 5C). The warts may have either a cuticular origin or they may develop from protrusions of the pectin layer of the cell wall covered by the cuticle (inset).

Conclusions

From the evidence presented here, it would appear that the various morphological types of glandular hairs in *P. scabrum* do not represent different trichome types but different developmental stages of a single glandular hair type. This may possibly have implications in the taxonomy of *Pelargonium* with the result that the number of trichome types distinguished (Oosthuizen 1983) will probably be reduced after further investigation of this aspect in other species.

Acknowledgements

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